بسم الله الرحمن الرحيم

**Sudan University of Sciences and Technology**
College of Graduate Studies

# Effect of Storage Period on CBC in Whole Blood Bags in Blood Bank that Containing CPDA-1 as Anticoagulant in East Nile locality

# تأثير فتره تخزين الدم على تعداد الدم الكلي في أكياس الدم الكامل في بنك الدم التي تحتوي على CPDA-1 كمانع تجلط في محلية شرق النيل

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## الآية

قَالَ تَعَالَىٰ:

﴿ يَٰٓأَيُّهَا ٱلنَّاسُ إِن كُنتُمۡ فِي رَيۡبٖ مِّنَ ٱلۡبَعۡثِ فَإِنَّا خَلَقۡنَٰكُم مِّن تُرَابٖ ثُمَّ مِن نُّطۡفَةٖ ثُمَّ مِنۡ عَلَقَةٖ ثُمَّ مِن مُّضۡغَةٖ مُّخَلَّقَةٖ وَغَيۡرِ مُخَلَّقَةٖ لِّنُبَيِّنَ لَكُمۡۚ وَنُقِرُّ فِي ٱلۡأَرۡحَامِ مَا نَشَآءُ إِلَىٰٓ أَجَلٖ مُّسَمّٗى ثُمَّ نُخۡرِجُكُمۡ طِفۡلٗا ثُمَّ لِتَبۡلُغُوٓاْ أَشُدَّكُمۡۖ وَمِنكُم مَّن يُتَوَفَّىٰ وَمِنكُم مَّن يُرَدُّ إِلَىٰٓ أَرۡذَلِ ٱلۡعُمُرِ لِكَيۡلَا يَعۡلَمَ مِنۢ بَعۡدِ عِلۡمٖ شَيۡـٔٗاۚ وَتَرَى ٱلۡأَرۡضَ هَامِدَةً فَإِذَآ أَنزَلۡنَا عَلَيۡهَا ٱلۡمَآءَ ٱهۡتَزَّتۡ وَرَبَتۡ وَأَنۢبَتَتۡ مِن كُلِّ زَوۡجِۭ بَهِيجٖ ٥ 5

صَدَقَ اللهُ العَظِيمُ

**سورة الحج الآية {5}**

## Dedication

To my family father, mother and my husband who are always beside me.

To my friends and colleagues.

To all peoples who participated fully and help me a lot to achieve this work.

To everyone from whom we learned.

## Acknowledgements

First of all, all thanks to **Allah** for help us to complete this work, and special thanks to our supervisor **Professor. Shadia Abdalaati Omer** for directing and guiding me.

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## Abstract

This is analytical study conducted in Umdownban Hospital East Nile locality - Khartoum State during March to December 2019. To evaluate the effect of storage period on complete blood count in whole blood bag in blood bank which containing citrate phosphate dextrose -1 as anticoagulant. Forty healthy male blood donors, aged 27± years, who attended Umdownban Hospital during the study period, were enrolled in the study. Participation was voluntary and an informed verbal consent had been taken from each participant before commencement of the study. Physiological tests, ABO blood group and viral screening have been done as routine tests for donation, collected blood bags were placed at 4-6°C for one month, anticoagulated whole blood was taken from each bag at day one, after 15 days and 30 days of collection. Blood was analyzed by Mindray BC-3000 Plus autohematological analyzer. The result were analyzed by SPSS version 20. ANOVA test was performed. The mean values of RBCs Count, Hb and HCT were not affected by storage. MCV was significantly (P=0.000) increased with the length of the storage period and the highest value (97.2±9.55fL) was found at day thirty. A significant decreased in MCHC was observed among the three periods the lowest value was (27.2±3.11g/dl) found in day thirty. The two storage periods registered significantly higher values (P=0.000) for RDW.CV than day one, and no significant variation was found between the two periods. RDW.SD% (P=0.014) value of day 30 was significantly higher than that of day one.

Day 15 and 30 registered significantly lower values for WBCs than day one, and no significant variation was found between them. The lymphocytes count was significantly (P=0.000) increased with the length of the storage period and the highest value (73.6±17.65%) was found at day thirty. The granulocytes count was significantly (P=0.000) decreased with storage and the lowest value (26.5±14.78%) was found at day fifteen.

Platelet count was significantly decreased with the length of the storage periods and the lowest value was (121±54.2× $10^9$cell/ L) registered in day thirty. PDW value of day thirty was significantly higher than that of day one. PCT value of day 30 was significantly lower than that of day one. No significant variation was found between day 15 and 30 with regard to PDW or PCT. MPV was not affected significantly ($P \leq 0.05$) with the storage. It is concluded that storage significantly effects some parameters of the CBC and the effect increases with the length of the storage periods.

## الخلاصة

هذه دراسة تحليلية أجريت في ولاية الخرطوم محلية شرق النيل تحديداً في مستشفى أم ضواًبان في الفترة مابين مارس الى ديسمبر 2019 لتحديد مدى تأثير فترة تخزين الدم على تعداد الدم الكلي في أكياس تحتوي على سترات فوسفات سكر العنب الأدنين كمضاد للتجلط. أربعون متبرع من الذكور الملائمين لمقياس مواصفات التبرع بالدم متوسط أعمارهم هو 27 عام الذين حضروا مستشفى ام ضوابان خلال فترة الدراسة. تم أخذ الدم منهم بعد موافقتهم لأغراض البحث قبل البدء في تسجيلهم للدراسة. تم إجراء الفحوصات الفسيولوجية وفحص فصائل الدم الرئيسية وفحص الفيروسات كإختبارات دورية عند اتبرع بالدم. ثم تم حفظ الدم في درجة حرارة 4–6 درجة مئوية لمدة شهر. تم أخذ عينة من الدم المحفوظ في اليوم الأول واليوم الخامس عشر واليوم الثلاثون. ثم تم عمل إختبار تعداد الدم الكلي بإستخدام جهاز Mindary BC–3000 Plus بعد إنتهاء فترة التخزين وأخذ النتائج تم تحليلها بإستخدام برنامج الحزم الأحصائية للعلوم الإجتماعية المحوسب الإصدار 20 وتم إختيار إختبار تحليل التباين في إتجاه واحد.

أظهرت النتائج أنه لا يوجد تأثير على الهيموقلوبين، عدد خلايا الدم الأحمر، حجم الخلايا المكدسة HCT خلال فترة التخزين. ولكن سجلت زيادة معنوية في متوسط كريات الدم الحمراءMCV (P=0.000) حيث سجلت اعلى قيمة (97.2±9.55fL) في اليوم الثلاثون. ونقصان معنوي في متوسط كتلة الهيموقلوبين في كرية الدم الحمراء MCHC (P=0.000) حيث سجلت ادنى قيمة (27.2±3.11g/dl) في اليوم الثلاثون. كما سجلت زيادة معنوية (P=0.000) في نسبة توزيع كريات الدم الحمراء RDW.CV و (P=0.014) في توزيع كريات الدم الحمراء RDW.SD في اليوم الثلاثون اعلى من اليوم الاول.

نقصان معنوي (P=0.000) في كريات الدم البيضاء بين اليوم الأول واليوم الخامس عشر ونقصان غير معنوي بين اليوم الخامس عشر واليوم الثلاثون. أيضا سجل نقصان معنوي (P=0.000) في الخلايا المحببة وسجلت زيادة غير معنوية بين اليوم الخامس عشر واليوم الثلاثون حيث سجلت ادنى قيمة (26.5±14.78%) في اليوم الخامس عشر. ولكن سجلت زيادة معنوية (P=0.000) في الخلايا الليمفاوية خلال فترة التخزين حيث سجلت اعلى قيمة (73.6±17.65%) في اليوم الثلاثون.

سجل نقصان معنوي (P=0.000) في الصفائح الدموية حيث سجلت ادنى قيمة ($10^9$ Cell/L × 121±54.2) في اليوم الثلاثون. بينما لم يتأثر متوسط حجم الصفائح الدموية MPV خلال فترة التخزين. لكن سجلت زيادة معنوية في قياس توزيع الصفائح الدموية PDW (P=0.001) ونقصان معنوي في حجم الصفيحات المكدسة PCT (P=0.000) هذا يلخص تأثر معنوي في بعض عوامل تعداد الدم الكلي مع زيادة فترة التخزين.

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## List of abbreviations:

| Abbreviation | Full text |
|---|---|
| 2,3DPG | 2,3Diphosphoglycerate |
| AABB | American Association of Blood Banks |
| ACD | Acitate Citrate Dextose |
| ADP | Adenosine Diphosphate |
| ANOVA | Analysis of Variance |
| APCs | Antigen Presenting Cells |
| AS | Additive Solution |
| ATP | Adenosine Triphosphate |
| CBC | Complete Blood Count |
| CDC | Central for Disease Control |
| CMV | Cytomegalo Virus |
| CP2D | Citrate Phosphate double Dextrose |
| CPD | Citrate Phosphate Dextose |
| CPDA-1 | Citrate Phosphate Dextrose Adenine-1 |
| CQI | Continuous Quality Improvement |
| CRYO | Cryopreciptatedantihemophilic factor |
| DEHP | Di-ethylehexylphthalate |

| | |
|---|---|
| EVF | Erythrocyte Volume Fraction |
| FDA | Food and Drug Association |
| FFP | Fresh Frozen Plasma |
| GMP | Good Manufacturing Practices |
| GVHD | Graft Versus Host Diseas |
| HbA | Adult Hemoglobin |
| HbF | Fetal Hemoglobin |
| HCT | Hematocrit |
| HES | Hydroxyethyl Starch |
| Hgb | Hemoglobin |
| HLA | Human Leukocyte Antigen |
| HPA | Human Platelet Alloantigen |
| IgA | Immunoglobulin A |
| IL | Interlukin |
| LDC | Liquid Crystal Display |
| LRRC | Leukocyte Reduced Red Cells |
| MCH | Mean Corbuscular Hemoglobin |
| MCHC | Mean Corbuscular Hemoglobin Concentration |
| MCV | Mean Corbuscular Volume |

| | |
|---|---|
| mmHG | Millimeter of Mercury |
| MPV | Mean Platelet Volume |
| NTBI | Non-Transferrin Bound Iron |
| PAGGSM | Phosphate Adenine Glucose Guanosine Saline Mannitole |
| PCT | Plateletcrit |
| PCV | Packed Cell Volume |
| PDW | Platelet Distribution Width |
| Pg | Picogram |
| PRBC | Packed Red Blood Cell |
| PRP | Platelet Rich Plasma |
| PTP | Post Transfusion Purpura |
| PVC | Polyvinyle Chloride |
| QA | Quality Assurance |
| QC | Quality Control |
| QSE | Quality System Essentials |
| RBCs | Red Blood Cells |
| RDW | Red cell Distribution Width |
| RDW.SD | Standard Deviation |
| RDW.CV | Coefficient of Variation |

| | |
|---|---|
| RNA | Ribonucleic Acid |
| ROS | Reactive Oxygen Species |
| SAG | Saline Adenine Glucose |
| SAGM | Saline Adenine Glucose Mannitol |
| sFasL | Serum Soluble Fas Ligand |
| SPSS | Statistical Package for the Social Sciences |
| TAGVD | Transfusion Associated Graft Versus Host Disease |
| TQM | Total Quality Management |
| TRALI | Transfusion Related Acute Lung Injury |
| WB | Whole Blood |
| WBC | White Blood Cells |
| WHO | World Health Organaization |

# Chapter I
# Introduction

## 1.1 Introduction:

Blood transfusion is the process of transferring blood or blood products from a donor into the circulating system of a recipient. Blood processing is defined as a series of processes to prepare components that will improve the hematological status of the patient. Anticoagulants and preservatives initially prevent clotting and thereafter maintain cell viability and function during storage. Citrate, phosphate, dextrose, and adenosine (CPDA-1) are usually used when the collected donation is to be stored as whole blood. Whole blood after collection can be immediately placed at +4°C ± 2°C (Hardwick, 2008).

Complete blood counts are done to monitor overall health, to screen for some diseases, to confirm a diagnosis of some medical conditions, to monitor a medical condition, and to monitor changes in the body caused by medical treatments (Ali, 2017).

Changes that alter the physiological properties occur in collected blood over time, and this is known as storage lesion. Coagulation factor activity (including factor VIII) deteriorates very rapidly in whole blood, particularly after the first 24 hours of storage and become not suitable for treatment of haemostatic disorders. Platelets in whole blood lose viability and functionality very quickly and will not be a suitable source for treatment of patients requiring platelet therapy. The red cells increase their affinity for oxygen and lose some viability. Leucocytes deteriorate with the release of leucocyte proteases (Queen *et al*., 2014).

After the first 2 weeks of storage, the haemostatic function of WB may vary and supplementation with fresher whole blood units or blood components, especially platelets (PLTs), may be necessary to promote hemostasis (Anderw *et al*., 2018).

RBCs transfusion is associated with increased mortality, serious infection and multiorgan failure, which may increase with prolonged RBCs storage before transfusion .Stored RBCs delivers large amount of iron to the monocyte and macrophage system. The insult induced by transfusion of stored RBC synergizes with subclinical overt signs and symptoms. Increased circulating iron, especially non-transferrin bound iron, enhances proliferation of certain pathogens (Eldad *et al*., 2010).

To avoid these problems, it is recommended to use blood component, and component use also permits optimal storage conditions for each of the components of blood, minimizes hemolytic reactions and supports precision treatment (Anderw *et al*., 2018).

**1.2 Rationale:**

Blood storage is important for emergency transfusions during surgery and for the treatment of anemia. Every day blood is required in hospitals in Sudan, The functionality and viability of stored human red blood cells is an important clinical issue in transfusion. Some morphological, count and biochemical alteration from long storage periods affect the functions of RBCs.

Storage of blood induces progressive biochemical and biomechanical changes that affect red cell viability, deformability, oxygen carrying capacity and microcirculatory flow. Blood bank of Umdownban Hospital receives blood every day. This hospital like most hospitals in Sudan use whole blood for transfusion. This study was performed, for the first time in Umdownban Hospital, to contribute to the existing information on the subject from the different hospitals of the Sudan.

## 1.3 Objectives:

### 1.3.1 General objective:

To evaluate effect of storage period on CBC in Whole blood bag containing CPDA-1 anticoagulant.

### 1.3.2 Specific objectives:

1-To determine effect of storage period on Hb, Hct , RBCs count and RBCs indices.

2-To determine effect of storage period on leukocyte count, lymphocytes and granulocytes.

3-To determine effect of storage period on platelets count and platelet indices.

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# CHAPTER II
# LITERATURE REVIEW

## 2.1 Blood:

Blood is composed of tow portion plasma and cells, plasma is a pale yellow fluid forms about 55% of blood volume and contains water (95%) and many solutes, including proteins, mineral ions, organic molecules, hormones, enzymes, products of digestion, and waste products for excretion in which are suspended red cells, white cells, and platelets. Blood flows through every organ of the body, it is kept in continuous circulation by the pumping action of the heart, flowing through arteries which carry the oxygenated blood from the heart to all parts of the body, and veins which carry the deoxygenated blood from the different parts of the body back to the heart and to the lungs. The arteries divide into smaller vessels called capillaries forming the capillary, or peripheral, circulation which supplies oxygen to the tissues. The capillaries rejoin to form the veins. The same amount of blood that is pumped out of the heart returns to it. There are about 5–6 liters of blood in the circulatory system of an adult and about 300 ml of blood in the system of a newborn infant (Cheesbrough, 2006).

### 2.1.1 Erythrocyte (red blood cells):

RBCs are produced in the bone marrow with the help of metals (iron, cobalt, manganese) vitamins (B12, B6, C, E, folate, riboflavin, pantothenic acid, thiamine), and amino acids. Erythropoiesis is regulated by erythropoietin, thyroid hormone, and androgens. The biconcave erythrocyte is thinner in the middle, creating a central pallor on blood. Such a cell possessing the normal amount of hemoglobin is called normochromic. The mature red blood cell carries oxygen attached to the iron in hemoglobin. Assessment of the RBC is to check for anemia and to evaluate normal erythropoiesis (Lokwani, 2013).

The normal RBC count is approximately 4.5 to 6 million cells per microliter. The parameters by which erythrocytes are usually measured are the blood hemoglobin (Hgb) in grams per deciliter (g/dL), the hematocrit (Hct) or packed cell volume (volume of RBCs as a percent of total blood volume), and the RBC count (millions of cells per μL). The size of red cells is measured as the mean corpuscular volume (MCV), reported in femtoliters. Red cells have a life span of approximately 120 days. Young red cells can be identified because they contain ribonucleic acid (RNA) with special stains such as new methylene blue, the RNA aggregates invisible particles called reticulin, young RBCs containing RNA are designated as reticulocytes, and the number of is the best estimate of RBC production. Reticulocytes cannot be specifically identified on the usual blood smear stains, but they stain slightly more blue than older RBCs (William, 2002).

**2.1.1.1 Haemoglobin:**

Each molecule of haemoglobin contains four linked polypeptide (globin) chains which in an adult consist of two alpha (α) chains containing 141 amino acids and two beta (β) chains containing 146 amino acids. Each polypeptide chain is combined with an iron containing porphyrin pigment called haem which is the oxygen carrying part of the haemoglobin molecule.

(Cheesbrough, 2006).

Each of these types of hemoglobins has a specific arrangement of globin chains and each globin chain is under the influence of a specific chromosome. Hemoglobin F (α2γ2), fetal hemoglobin, begins to be synthesized at approximately 3 months in fetal development and remains as the majority hemoglobin at birth. Between 3 and 6 months post delivery, the amount of gamma chains declines and the amount of beta chains increases, making hemoglobin A (α2β2) the majority adult hemoglobin, 95% to 98%. Hemoglobin A2 (α2δ2), 1% to 3%, and

hemoglobin F less than 1%, are also part of the normal adult hemoglobin complement (Ciesla, 2007).

**2.1.1.2 Erythrocyte indices:**

The indices are calculated from the erythrocyte count, hemoglobin concentration, and hematocrit. With the advent of automation in hematology, the erythrocyte indices are measured and/or calculated from data collected by erythrocyte analysis. The electrical impedance counters measure the MCV by averaging the heights of the voltage pulses. The MCH, MCHC, and hematocrit are calculated from the measured values, MCV, hemoglobin, and erythrocyte count. The erythrocyte indices are used in the morphologic classification of anemias, defining normocytic, microcytic, and macrocytic anemias. The normal MCV is ~80 to 100 fL. Red blood cells that are smaller than 80 fL are called microcytic; those that are larger than 100 fL are called macrocytic (McKenzie and Williams, 2010).

**2.1.1.3 Hematocrit-packed cell volume (PCV):**

The hematocrit (Hct) or packed cell volume (PCV) or erythrocyte volume fraction (EVF) is the ratio of the volume of erythrocytes to that of the whole blood and is reported as percentage. The hematocrit is one of the most precise methods of determining the degree of anemia or polycythemia, increase or decrease in RBC concentration. Normal Range is 38.8–46.4 % for males and 35.4–44.4 % for females. The manual method of measuring Hct has proved to be a simple and accurate method of assessing red cell status. It is easily performed with little specialized equipment. The spun Hct measures the red cell concentration, not red cell mass (Lokwani, 2013).

**2.1.1.4 Reticulocyte count:**

Reticulocytes are immature nonnucleated erythrocytes containing residual RNA, in the erythrocyte maturation sequence, the reticulocyte spends about two

days in the bone marrow and one day in the peripheral blood. As the reticulocyte matures, the amount of RNA decreases, the quantitation of reticulocytes present in the peripheral blood provides a method of evaluating the bone marrow's erythropoietic activity, using a supravital stain (new methylene blue), residual ribosomal RNA is precipitated within the reticulocytes. A blood smear is prepared from the mixture of anticoagulated whole blood and supravital stain, the smear is examined microscopically using the oil immersion lens (1000× magnification) fitted with a field-restricted ocular, an erythrocyte containing two or more particles of blue stained material is a reticulocyte. The number of reticulocytes is expressed as a percentage of the total number of erythrocytes counted (McKenzie and Williams, 2010).

**2.1.1.5 Red cell distribution width:**

RDW expresses the coefficient of variation of the erythrocyte volume distribution. The RDW is calculated by dividing the SD by the mean of the red cell size distribution. The RDW is expressed numerically as the coefficient of variation percentage. The normal range is 11.5% to 14.5% (Turgeon, 2012).

**2.1.2 Leukocyte (white blood cells):**

Several types of leukocytes, or white blood cells (WBCs) are found in the blood. The normal WBC count is ~4,000 to 10,000/µL (4.0–10.0 ×103/µL). Leukocytes are usually divided into granulocytes, which have specific granules, and agranulocytes, which lack specific granules.

Granulocytes are divided into neutrophils (with faintly staining granules), eosinophils (with large reddish or eosinophilic granules), and basophils (with large dark blue or basophilic granules). Agranulocytes are divided into lymphocytes and monocytes. Although they are called white blood cells, leukocytes predominantly function in tissues. They are only in the blood transiently, while they travel to their site of action (William, 2002).

### 2.1.2.1 Differential leukocyte count:

Differential leukocyte analysis is a fundamental part of hematology because of the major implications it has for the diagnosis and follow -up of hematologic diseases such as acute and chronic leukemias, myeloproliferative and lymphoproliferative disorders, and myelodysplastic syndromes. Differential leukocyte analysis has for a long time been performed using only microscopic examination of the bone marrow or peripheral blood, Various types of automated processes, based on different principles, were then developed, which were designed to provide reliable leukocyte counts and proper normal differentials. Immunologic principles and multiparameter flow cytometry have already been applied to the study of leukocyte maturation (Marchant and Davis, 2012).

The differential count, expressed as the percentage of each type of cell, should be related to the total leucocyte count and the results should be reported in absolute numbers ($\times 10^9$/l). Myelocytes and metamyelocytes, if present, are recorded separately from neutrophils. Band (stab) cells are generally counted as neutrophils, but it may be useful to record them separately. They normally constitute <6% of the neutrophils; an increase may point to an inflammatory process even in the absence of an absolute leucocytosis. However, the band cell count is imprecise and, although it is sometimes recommended in infants, it has been found to be unhelpful in predicting occult bacteraemia in this group (Briggs and Bain, 2011).

### 2.1.3 Platelets (thrombocytes):

Platelets, occasionally called thrombocytes, are involved in hemostasis. Human blood platelets are small, anucleated cells that play a critical role in hemostasis and thrombosis. They have pale blue cytoplasm with reddish-purple granules. They are derived from megakaryocytes in the bone marrow by release of fragments of megakaryocyte cytoplasm. Human platelets normally circulate for approximately 10 days, constantly surveying the integrity of the vessel wall. Normal human

platelets are small and discoid in shape (0.5 × 3.0 μm), have a mean volume of 7–11 fL, and normally circulate in relatively high numbers between 150 and 400 × 109/L (Key et al., 2009).

Platelets have different types of granules, designated alpha granules and dense bodies. Platelet granules contain clotting factors, adenosine diphosphate (ADP) and adenosine triphosphate (ATP), calcium, serotonin, and catecholamines, many of these stimulate platelet aggregation or are important in the coagulation cascade. Senescent platelets are removed by the spleen (William, 2010).

In addition to their important role in haemostasis and thromposis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defence, wond healing angiogenesis, and remodelling. Platelets mediate leukocyte movement from the blood stream through the vessel wall to tissue (Budak *et al.,* 2016).

**2.1.3.1 Platelet indices:**

Modern haematology analysers in routine diagnostic use, which measure platelet indices, use impedance counting or optical light scatter counting techniques. Plateletcrit (PCT),mean platelet volume (MPV), and platelet distribution width (PDW). In MPV the analyser calculated measure of thrombocyte volume is determined directly by analyzing the platelet distribution curve, PDW is indicator of volume variability in platelets size and is increased in the presence of platelet anisocytosis, PCT is the volume occupied by platelets in the blood as percentage (Budak *et al.*, 2016).

**2.2 Cell counts:**

Cell counts are important parameters in evaluating the blood. Cell counts may be determined either manually or by automated hematology analyzers. Whether performed by manual or automated methodologies, the accuracy and precision of the counts depend on proper dilution of the blood sample. The type of diluent is

dependent on the cell type to be enumerated. Thus, red cell counts require dilution with an isotonic medium, whereas in white cell or platelet counts, a diluents that lyses the more numerous red cells is often used to simplify counting. Errors in cell counts are caused primarily by errors in sample measurement, dilution, or enumeration of cells. Manual counts are done using a microscope after appropriate dilution of the sample in a hemocytometer, a specially constructed counting chamber that contains a specific volume. Red cells, leukocytes, and platelets may be counted. Due to the inherent imprecision of manual counting and the amount of technical time required, most cell counting is now performed by automated instruments that increase the accuracy and speed of analysis by the clinical laboratory (Greer et al., 2014).

### 2.2.1 General principles of hematology automation:

Automated use several distinct technical approaches, including those that measure changes in electrical impedance and those that use differences in light scatter or optical properties, either alone or in combination. Another recent advancement in hematology analyzers is incorporation of argon laser technology, allowing integration of some flow cytometric data using specific fluorochrome stains. Each instrument presents a pictorial representation of the hematological data registered as either a histogram or a scatterogram (Lokwani, 2013).

Whereas the impedance principle uses low -voltage current to measure a cell's total volume, the radiofrequency method uses high -voltage electromagnetic current to measure a cell's nucleus. The alternating current in the radiofrequency range short -circuits the bipolar lipid layer of a cell's membrane, allowing the energy to penetrate the cell. This enables the collection of information proportional to cell size and internal structure, including chemical and physical composition and nuclear volume (Marchant and Davis, 2012).

## 2.3 Blood components:

It is possible to separate different components of blood from a single unit of whole blood. These components can be used individually to help more than one patient with many purposes. Thus, red cells can be transfused to an anemic patient and plasma for a burns patient. This also ensures that only the required components are transfused (Nayak et al., 2012).

### 2.3.1 Whole blood:

Whole blood contains RBCs and plasma, with a hematocrit level of approximately 38 percent. The appropriate storage temperature is 1_ to 6°C, and the shelf-life is dependent on the preservative used that of ACD and CPD is 21 days, and that of CPDA-1 is 35 days
(Harmening, 2005).

The initial step of component manufacturing of whole blood is centrifugation, which leads to sedimentation or packing of the iron-laden RBCs. Next the platelet-rich plasma is expressed, resulting in the RBC product. This product can then undergo a number of modifications, including leukoreduction, freezing, rejuvenation, washing, irradiation, and/or volume reduction (Shaz et al., 2013).

### 2.3.2 Red blood cell products:

Red blood cells (RBCs) are also known as packed RBCs (pRBCs) and red cells. The majority of RBC products are made from whole blood donated as 450 or 500 ml units into an anticoagulant-preservative solution, about 11% of RBC products are collected via automated RBC apheresis (Shaz et al., 2013).

RBCs are prepared by separating the RBCs from the plasma using a method that results in a final hematocrit level of less than or equal to 80 percent, depending on the anticoagulant and preservative used in collection. RBCs are useful in patients who require the increased RBC mass but may be at risk of circulatory

overload. RBCs can be prepared any time during the normal dating period by centrifugation or sedimentation. There is variation in the amount of plasma removed from the unit, depending on the anticoagulant-perservative solution used (Harmening, 2005).

### 2.3.3 Washed red blood cells:

Washing is typically performed by diluting a standard 350 mL RBC unit with 1-2 L of normal saline, and then concentrating the diluted RBCs back to the desired hematocrit, with a centrifuge or a centrifugation-based cell processor, and discarding the supernatant. Centrifugation, however, subjects stored RBCs to substantial mechanical damage, inducing hemolysis and increasing fragility of the cells (Voros et al., 2018).

### 2.3.4 Plasma:

Plasma in a unit of whole blood can be separated at any time during storage, up to 5 days after the expiration date of the whole blood. When stored frozen at –18 °C or colder, this component is known as plasma and can be used up to 5 years after the date of collection. If not frozen, it is called liquid plasma, which is stored at 1 to 6°C and transfused up to 5 days after the expiration date of the whole blood from which it was prepared. Fresh Frozen Plasma (FFP) is plasma prepared from whole blood, either from the primary centrifugation of whole blood into red cells and plasma or from a secondary centrifugation of PRP. The plasma must be frozen within eight hours of collection (Brecher, 2005).

### 2.3.5 Cryoprecipitate:

Cryoprecipitate ant hemophilic factor (CRYO) is the cold insoluble portion of plasma processed from FFP by cold precipitation. The precipitate is formed as FFP is thawed at 1 to 6□C. The stability of the coagulation factors is maintained for up to 1 year at –18□C storage. It is a plasma-derived product that contains the highest

concentration of fibrinogen, factor VIII, and a variable percentage of the original plasma concentration of von Willebrand factor and factor XIII (30%). CRYO is also used as a source of fibrinogen to form a fibrin glue or sealant (when added to thrombin) and is also indicated to ameliorate the platelet dysfunction in uremia. Like FFP, it contains ABO antibodies, requiring consideration of recipient red cell compatibility (Tenorio et al., 2007).

**2.3.6 Leukocyte-Reduced red cells:**

Leukocyte-reduced red cells (LRRCs) can be prepared by a variety of methods, resulting in differing degrees of white cell removal. The minimum standard, set by the American Association of Blood Banks (AABB), is a leukocyte number in the final component of $<5 \times 10^8$. Early techniques of preparation involved centrifugation or washing with saline, whereby the buffy coat was repeatedly removed. Subsequently, a second-generation technique known as the spin-cool-filter method was introduced. This required use of 1-week-old red cells, which were centrifuged and then cooled for 4 hours to enhance microaggregate formation before passage through a microaggregate filter. Currently the most widely used method of leuko-reduction is filtration. Most recently, blood bags with in-line filters, which allow prestorage leuko-reduction, have become available. The major indication for the use of LRRCs has been the prevention of the nonhemolytic febrile transfusion reaction, A second important indication for LRRCs is the prevention of alloimmunization to HLA antigens that can adversely affect post-transfusion platelet increments, such as in cancer patients undergoing chemotherapy (Hofman *et al*, 2000).

**2.3.7 Granulocyte concentrates:**

These are prepared as buffy coats or on blood cell separators from normal healthy donors or from patients with chronic myeloid leukaemia. They have been used in patients with severe neutropenia, who are not responding to antibiotic

therapy but it is not usually possible to give sufficient amouts. They may transmit CMV infection and must be irradiated to eliminate the risk of causing GVHD (Hoffbrand, *et al*., 2006).

### 2.3.8 Irradiated blood:

The major technology for preventing TA-GVHD is irradiation of blood components to inactivate residual lymphocytes. Gamma or X-irradiation of blood components, by validated systems, is the recommended procedure to prevent TA-GVHD (Treleaven et al., 2010).

### 2.3.9 Platelet concentrate:

Platelet concentrate may be obtained from a single donor or pooled plasma. Platelets can also be obtained from a single donor by platelet apheresis. They are stored at 20° to 24°C and have shelf-life of 5 days. Platelets have ABO antigens on their surface but do not express Rh antigen. But it is advisable to transfuse Rh negative persons with platelets only from Rh negative persons (Nayak et al., 2012).

## 2.4 Blood containers:

Blood must be collected into a container that is pyrogen-free and sterile and contains sufficient anticoagulant for the quantity of blood to be collected. The container label must state the type and amount of anticoagulant and the approximate amount of blood collected. Blood bags may be supplied in packages containing more than one bag. The manufacturer's directions should be followed for the length of time unused bags may be stored in packages that have been opened (Brecher, 2005).

Blood bag plasticizers appear to influence membrane stability. Red cells are stored in polyvinyl chloride (PVC) bags that contain the plasticizer di-2-ethylhexylphthalate (DEHP). Morphologic deterioration is greater in RBCs stored in containers that do not have DEHP, with increased hemolysis and loss of deformability, suggesting that DEHP has a direct membrane stabilizing effect.

Adding DEHP can both prevent and repair deterioration of stored red cells (Greer *et al*., 2014).

### 2.4.1 Anticoagulants in blood banks:

Citrate-phosphate-dextrose (CPD) became the standard anticoagulant for both WB and components. CPD-stored blood was granted a shelf life of 21 days (2–4°C). Then CPD supplemented with adenine (CPDA-1) was in use, increasing the available storage time of WB and RBCs to 35 days. Extensions in shelf life were attributed to adenine's contribution to the increased synthesis of adenosine triphosphate. Citrate–phosphate-double dextrose (CP2D) has been less commonly used as an anticoagulant for blood and blood products with the higher concentration of dextrose and thus higher osmolality than CPD intended to support RBCs. However, CP2Danticoagulated WB has the same 21-day shelf life as CPD (Meledeo *et al.,* 2019).

### 2.4.2 RBC additive solutions:

The first RBC additive solution, saline-adenine glucose (SAG) was developed by European researchers in the late 1970. Then the same researchers added mannitol to help protect the RBC membrane and reduce hemolysis, which enabled up to 6 weeks refrigerated storage of RBCs at a haematocrit of approximately 55 to 60 percent. This modified SAG formulation was named SAGM and to this day SAGM is the most widely used RBC additive solution. Other additive solutions, which are all essentially variations of SAG/SAGM, have since been developed and commercialised, including AS-1, AS-3, AS-5, MAP and PAGGSM. These solutions tend to provide improved preservation of RBCs compared to RBCs stored in SAGM, such as decreased haemolysis and reduced shedding of microparticles from the RBCs. Nevertheless, the specific biological mechanisms that are modulated in RBCs stored in the variant SAG/SAGM solutions have not been precisely identified (Sparrow, 2012).

## 2.5 Storage of blood components:

Red cell concentrates and platelet concentrates are stored as fresh products ready for transfusion, although in special circumstances can be frozen using specific cryopreservation procedures. Red cell concentrates are stored refrigerated (2–6 °C) for up to 42 days (maximum duration may vary depending on the type of additive solution used and/or local regulatory criteria). Platelet concentrates are stored at 20–24 °C for up to 5 days, with continuous gentle agitation to maintain maximum biological function. During storage, red cells and platelets continue to metabolise and undergo a range of physiochemical changes, collectively referred to as the storage lesion. The storage lesion ultimately affects the in vivo function and survival of transfused red cells and platelets and thus limits their shelf life. Plasma is rapidly frozen to <–25 °C in order to maintain maximum function of labile coagulation factors, such as Factor VIII (Greening et al., 2009).

## 2.6 RBC Storage lesion:

The RBC storage lesion is the term that collectively refers to a number of biochemical and physical changes occurring to the RBCs themselves, as well as the resultant changes in the entire RBC product during storage. When blood is stored in a liquid state there is a progressive loss of viability of the red cells. The purpose of modern anticoagulants and additive solutions is to reduce these changes to a minimum (Shaz et al., 2013).

Decreased RBC viability is correlated with lesion of storage that is associated with various biochemical changes including decreased pH, a buildup of lactic acid, a decrease in glucose consumption, a decrease in adenosine triphosphate levels and a loss of RBC functions. When hemoglobin value decreases during storage because of hemolysis of RBC then Methemoglobin and sulfhemoglobin values are similarly reduced (Bashi and Saleh, 2009).

During storage, red cells metabolize glucose, producing lactic and pyruvic acid, resulting in lower pH and decreased glycolysis. As glycolysis slows RBC ATP content falls. Because human RBCs contain no enzymes to synthesize adenine or other purines de novo the nucleotide pool gradually becomes exhausted (Greer *et al*., 2014).

The most evident changes affecting red blood cells during the storage period are alterations of the cell phenotype, which varies from a smooth discoid shape to a phenotype characterized by various membrane protrusions (echinocyte) and finally to a spheroid-shaped cell (spheroechinocyte). The storage lesion also involves the fluxes of sodium ions (massive entry into the cell) and potassium ions (exit from the cell), since the Na+/K+ pump is inactive at 4°C. Another biochemical effect is a clear decrease in the levels of 2, 3-DPG (which is consumed already within the first week), translating into increased affinity of haemoglobin for oxygen and consequently decreased capacity of the red blood cells to release oxygen according to local metabolic needs. Various irreversible events occur during the storage process including fragmentation and aggregation of proteins and lipids activated by radical species generated by prolonged continuous oxidative stress (Alessandro, 2010).

**2.7 Donor selection:**

The donor screening process is one of the most important steps in protecting the safety of the blood supply. The process is intended to identify elements of the medical history and behavior or events that put a person at risk for transmissible disease or at personal medical risk. Donor selection is based on a medical history and a limited physical examination done on the day of donation to determine if giving blood will harm the donor or if transfusion of the unit will harm a recipient (Brecher, 2005).

### 2.7.1 Criteria for blood donor selection:

Only individuals in good health should be accepted as blood donors. Donors should feel well on the day of donation and be able to perform their routine daily activities. The low age limit for blood donation in most countries is 18 years. Upper age limits between 60 and 70 years. A donor should weigh at least 45 kg to donate 350 ml (± 10%) or 50 kg to donate 450 ml (± 10%). A normal pulse rate of 60-100 per minute and body temperature not more than 37.6°C. A normal blood pressure (systolic 120-129 mmHg, diastolic 80-89 mmHg) (WHO, 2012).

### 2.7.2 Registration:

Blood collection facilities must confirm donor identity and link the donor to existing donor records. The following is a list of information used by the collection facility in the registration process and is kept on record by use of a single donation record form name, date and time of donation, address, telephone, gender and age or date of birth (Harmening, 2005).

### 2.7.3 Donor‘s consent:

Prior to each donation, the donor must sign a written informed consent for collection of the blood. The donor must have the freedom to ask questions receive answers to these questions and finally, the option to consent or refuse to participate in donation. Each donor is educated on risks of the procedure and infectious disease (Whitlock, 2010).

### 2.7.4 Medical history questionnaire:

Obtaining an accurate medical history of the donor is essential to ensure benefit to the recipient. The medical history questions have been developed and revised as necessary by the AABB and FDA. The interviewer should be familiar with the questions and the interview should be conducted in a secluded area of the blood center. The questions are designed so that a simple “yes” or “no” can be answered

but elaborated if indicated. The medical history is conducted on the same day as the donation (Harmening, 2005).

### 2.7.5 Autologous donors:

An autologous donor is one who donates blood for his or her own use; thus, such a donor is referred to as the donor patient. Autologous blood is safer than allogeneic blood. There is no risk of disease transmission, transfusion reactions, or alloimmunization to white blood cells, RBCs, platelets, or plasma proteins. Autologous blood transfusion is a viable and common alternative therapy for many patients undergoing transfusion. There are four different types of autologous donation is preoperative collection, acute normovolemic hemodilution, intraoperative collection and postoperative collection (Harmening, 2005).

### 2.7.6 Donor deferral:

Donor who do not to meet the selection criteria should be deferred on a temporary or permanent basis. All deferred donors should be treated with respect and care in a confidential manner and should be given a clear explanation of the reason for deferral .Temporarily deferred donors should be advised on when they could donate and encouraged to return. Donors are less likely to return to donate blood if unclear or unsatisfactory information is given about the reason for deferred (WHO, 2012).

## 2.8 Transfusions and the immune response:

Transfusions affect both the innate and the adaptive immune system. Transfusions contain many foreign antigens and always, increasing with longer storage intervals, apoptotic, and necrotic cells. Immediately after blood withdrawal, granulocytes start to deteriorate, followed by macrophages, although viable lymphocytes can be detected after more than 25 days of storage. Apoptotic cells, expressing annexin V or phosphatidylserine, immediately engage a ligand on macrophages which start to produce anti-inflammatory cytokines such as

prostaglandin E-2 and transforming growth factor-beta during storage, soluble response modifiers accumulate in blood products. Factors derived from leukocytes are elastase, soluble HLA class I and II molecules, sFasL, and proinflammatory cytokines IL-1, IL-6, and IL-8. Pro inflammatory cytokines are an importantcause of nonhemolytic febrile transfusion reactions and in particular IL-8 is associated with transient posttransfusion leukocytosis, observed in critically ill patients. Beside gradual apoptosis, functional lesions occur during storage. After 3–5 days of storage, the responder capacity of T cells decreases owing to a phosphorylation defect, impairing the protein synthesis of T cells upon signaling of the T cell receptor. This reduces the proliferative response of donor lymphocytes, relevant to impaired transfusion associated graft-versus-host disease. The stimulator capacity by donor APCs of the direct pathway is irreversibly abrogated after 10–14 days of storage because of loss of costimulatory molecules (Maniatis, et al., 2011).

### 2.8.1 Red cell antigens and blood group antibodies:

Approximately 400 red blood cell group antigens have been described. The clinical significance of blood groups in blood transfusion is that individuals who lack a particular blood group antigen may produce antibodies reacting with that antigen which may lead to a transfusion reaction. The different blood group antigens vary greatly in their clinical significance with the ABO and Rh (formerly Rhesus) groups being the most important (Hoffbrand, et al., 2006).

### 2.8.2 Clinical significance of blood group antigens:

The importance of blood groups in clinical medicine lies in the fact that an antigen may in certain circumstances react with its corresponding antibody and cause harmful clinical effects. Of the many red cell blood group systems only two are of major clinical importance the ABO and Rh systems. The other systems are of much less clinical importance since, naturally occurring antibodies are found

only occasionally and when present usually react only at low temperatures and immune antibodies are formed only occasionally because many of the antigens are of low antigenicity. Some are strongly antigenic (e.g. Kell), but are of low frequency and therefore the chances of immunization are relatively small. The harmful clinical effects of red cell antigen-antibody reactions are haemolytic transfusion reactions, and haemolytic disease of the newborn. Haemolytic transfusion reactions are most often due to incompatibilities involving the ABO and Rh systems and only rarely the other systems (Frikin et al., 2006).

### 2.8.3 Compatibility testing (Pretransfusion testing):

Before transfusion of any blood or its components, it is essential to know whether they are compatible with the recipient's blood. This is achieved by performing a set of procedures known as compatibility testing. Sometimes the term compatibility test and cross matching are used interchangeably, but cross-match is only a part of compatibility test.

Compatibility tests include:

- Review of patient's past blood bank history and records (if done earlier).
- ABO and Rh typing of the recipient and donor.
- Antibody screening test of recipient's and donor's serum.
- Cross-matching (Nayak *et al*., 2012).

## 2.9 Transfusion reactions:

A transfusion reaction is defined as any unfavorable response in a blood or blood product recipient. Transfusion reactions display a range of symptoms from mild exacerbation up to and including death. Transfusion reactions can be divided into categories. The most common divisions are acute vs. delayed and immune vs. non-immune. A synopsis of the categories

Includes: Acute and delayed: Acute reactions occur in a short time frame from the initiation of the transfusion. Delayed reactions occur hours, days, or (rarely)

weeks after the completion of the transfusion. Also to hemolytic vs. non-hemolytic: Hemolytic reactions are those that result in the destruction of red blood cells. The hemolysis may result from an immune process or physical or chemical damage to the red cells. Non-hemolytic reactions do not involve the destruction of red cells (Whitlock, 2010).

**Table 2.1: Transfusion reactions:**

| **Acute Immune Mediated Reactions** |
|---|
| Hemolytic<br>Febrile<br>Anaphylactic<br>Urticaria<br>TRALI (Transfusion-Related Acute Lung Injury) |
| **Acute Non-Immune-Mediated** |
| Circulatory Overload<br>Bacterial Contamination<br>Physical Red Blood Cell Damage |
| **Delayed Immune Medicated Reactions Hemolytic** |
| Graft vs. Host Disease (GVHD)<br>Post-transfusion Purpura (PTP) |
| **Delayed Non-Immune Mediated** |
| Disease Transmission<br>Hemosiderosis (Iron Overload) |

(Whitlock, 2010).

### 2.9.1 Immune causes of non-hemolytic transfusion reactions:

#### 2.9.1.1 Allergic reactions:

Symptoms may either occur within seconds or minutes of the start of transfusion or may take several hours to develop. Most reactions are mild and are usually associated with cutaneous manifestations such as urticaria, rash, pruritis and flushing (Domen *et al.*, 2003).

#### 2.9.1.2 Anaphylaxis:

Anaphylaxis is a more form of an allergic reaction in which severe hypotension, shock, and loss of consciousness may occur. Anaphylaxis is commonly seen in IgA deficient recipients where it is caused by antibodies against donor IgA. The term anaphylactoid is used for reactions with symptoms similar to anaphylaxis but which are not mediated by IgE (Sahu et al., 2014).

#### 2.9.1.3 Febrile reactions:

A febrile reaction is one of the most common transfusion reactions. A febrile reaction is an increase of at least 1°C in temperature during the course of the transfusion, and is caused by antibodies to antigens on white cells or platelets. The recipient exhibits fever and chills. The symptoms are attributed to pyrogenic cytokines and intracellular contents released from donor leukocytes or infused with the blood component. These substances react with leukocyte reactive antibodies produced by the recipient. A febrile transfusion reaction is not life threatening, treatment should include termination of the transfusion and treatment of the symptoms (Whitlock, 2010).

#### 2.9.1.4 TRALI:

TRALI is a life-threatening complication of transfusion which may have a very dramatic clinical presentation indistinguishable from adult respiratory distress syndrome. In most cases it begins within 2 hours of transfusion but may be up to 4 or 6 hours following administration of a plasma containing blood component.

Symptoms generally include fever, hypotension, chills, cyanosis, nonproductive cough, and dyspnea. The precise mechanism involved in the development of TRALI is not clear, but two possible mechanisms have been postulated, an antibody mediated and a soluble mediator-mediated. These mechanisms both involve the activation of granulocytes and the triggering of an inflammatory process, leading to the sequestration of neutrophils in the lung. In the vast majority of cases, investigators have demonstrated the presence of HLA class I and class II or granulocyte-specific antibodies in the donor. In about half the cases studied, the HLA antibodies in the implicated donor correspond with one or more of the HLA antigens in the recipient. In some cases clinically diagnosed as TRALI, no antibody has been identified. It has been suggested that in these cases the granulocyte activation is mediated by a soluble lipid substance, which accumulates during the storage of the products (Maniatis, et al., 2011).

**2.9.1.5 GVHD:**

Is an under-diagnosed condition in clinical practice, it can occur in immunocompromised as well as immunocompetent host. Transfusion associated graft versus host disease (TA-GVHD) is a serious complication of blood transfusion with high mortality rate. It occurs when immunologically competent lymphocytes from the donor are introduced into an immunoincompetent host who is unable to destroy the donor lymphocytes. The donor lymphocytes engraft, recognize the host as foreign and then attack host tissue (Sohi and Jacob, 2005).

**2.9.1.6 PTP:**

It occur between 1 and 24 days after transfusion. Patients typically present with purpuric rash and thrombocytopenia resulting in bleeding from mucous membranes and the gastrointestinal and urinary tracts. The primary cause of mortality is intracranial haemorrhage. Antibodies against HPA-1a are responsible in most cases although, antibodies to HPA-1b, other platelet antigens, and HLA antigens are also

implicated. The current treatment of choice for PTP is high dose intravenous immune globulin (Sahu et al., 2014).

### 2.9.2 Non-immune causes of transfusion complications:

#### 2.9.2.1 Bacterial contamination of blood products:

Although the frequency of bacterial contamination of blood components is low, this type of septic reaction can have a rapid onset and lead to death. Of the deaths caused by bacterial contamination of blood components reported to the centers for disease control (CDC), most are caused by blood components contaminated by Yersinia enterocolitica.. Cases have been reported with transfused RBCs, platelets, and other blood components as well as with manufactured products such as intravenous solutions and HES. Transfusion reactions attributed to bacterial contamination reactions are commonly caused by endotoxin produced by bacteria capable of growing in cold temperatures (psychrophilic) such as Pseudomonas species, Escherichia coli, and Y. enterocolitica (Harmening, 2005).

#### 2.9.2.2 Circulatory overload:

Transfusion has been associated with significant morbidity. Chronic transfusion results in accumulation of excess iron that surpasses the binding capacity of the major iron transport protein, transferrin. The resulting non-transferrin bound iron (NTBI) can catalyze the production of highly reactive oxygen species (ROS) leading to significant and wide spread injury to the liver, heart, and endocrine organs as well as increases in infection .The effects appear to be more profound with increasing age of stored blood . The progressive release of free iron associated with storage time suggests that morbidity following acute transfusion, like that seen in chronic transfusion, may be due in part to elevated levels of NTBI. It is clear that transfusion is necessary in many instances; however,

its risks and benefits must be carefully balanced before proceeding to avoid unnecessary iron toxicity (Ozment and Turi, 2009).

Another problem when large volumes of FFP, Whole Blood, or Platelets are transfused rapidly, particularly in the presence of liver disease, plasma citrate levels may rise, binding ionized calcium and causing symptoms. Hypocalcemia is more likely to cause clinical manifestations in patients who are in shock or are hypothermic. A decrease in ionized calcium increases neuronal excitability, leading, in the awake patient or apheresis donor, hypocalcemia is thought to increase the respiratory center's sensitivity to CO2, causing hyperventilation. Because myocardial contraction is dependent on the intracellular movement of ionized calcium, hypocalcemia also depresses cardiac function (Brecher, 2005).

**2.10 Quality control and quality assurance in the blood bank:**

Quality is at the forefront of all processes in the laboratory. Quality in the blood bank and transfusion service has all of the components of quality test performance .Its expanded focus includes the collection, testing, and issuing of blood components. Regulatory agencies have increased requirements for processing components and the use of good manufacturing practices (GMP). GMP is a series of procedures that blood banks follow as a part of quality assurance (QA) practices within the transfusion service. The use of extensive computer technology has expanded quality services in the laboratory. Blood bank quality systems should include a global view of quality as well as daily quality control testing. The American Association of Blood Banks (AABB) has developed ten guidelines that define the minimum items required for the maintenance of a quality system in the blood bank or transfusion service. These guidelines are the Quality System Essentials (QSE) (Whitlock, 2010).

## Table2.2: Infectious agents transmissible by blood transfusion:

| Viruses | Bacteria | Protozoa | Prions |
|---|---|---|---|
| **Hepatitis viruses**<br>*-Hepatitis A virus (HAV).*<br>*-Hepatitis B virus (HBV).*<br>*-Hepatitis C virus (HCV).*<br>*-Hepatitis D virus (HDV) (requires co-infection with HBV).*<br>*-Hepatitis E virus (HEV).*<br>**Retroviruses:**<br>*-Human immunodeficiency virus (HIV)* 1 and +2 (+ + other subtypes).<br>*-Human T-cell leukemia virus* (HTLV) I and II.<br>**Herpes viruses:**<br>*-Human cytomegalovirus (HCMV).*<br>*-Epstein–Barr virus (EBV).*<br>*-Human herpesvirus 8 (HHV-8).*<br>**Parvoviruses:**<br>*-Parvovirus B19.*<br>**Miscellaneous viruses:**<br>*-GBV-C [previously referred to as hepatitis G virus (HGV)]*.<br>*-TTV*<br>*-West Nile virus* | **Endogenous:**<br>*-Treponema pallidum* (syphilis).<br>*-Borrelia burgdorferi* (Lyme disease).<br>*-Brucella melitensis* (brucellosis).<br>*-Yersinia enterocolitica.*<br>-Salmonella spp.<br>**Exogenous**: (environmental species and skin commensals):<br>*-Staphyloccocal spp.*<br>*-Pseudomonads.*<br>*-Serratia spp.*<br>*-Rickettsiae: Rickettsia rickettsii (Rocky Mountain* spotted fever).<br>*-Coxiella burnettii* (Q fever). | *-Plasmodium spp.* (malaria).<br>*-Trypanosoma cruzi* (Chagas disease).<br>*-Toxoplasma gondii* (toxoplasmosis).<br>*-Babesia microti/divergens* (babesiosis)<br>*-Leishmania spp.* (leishmaniasis). | *-Variant Creutzfeldt–Jakob disease (vCJD).* |

(Maniatis, *et al.*, 2011).

## 2.11 Previous studies:

Prospective cohort study was conducted at Hayatabad Medical Complex Peshawar .collected blood from 40 health donors in CPDA-1 anticoagulant at 2_8°C for 30 days. Study showed that variations occurred both in hematological and biochemical parameters. Significant changes were observed in RBC, Hb, MCHC, WBC and platelet count. No significant changes were observed in MCV and MCH. In biochemical parameters significant increase in potassium, significant decrease in sodium and chlorides. Therefore, for cardiovascular and renal failure patients fresh blood would be recommended to avoid adverse transfusion reaction (Asif zeb M.,et al, 2018).

The study was conducted in L. N. Medical college and J. K. Hospital, Bhopal, blood was drawn from 30 healthy volunteer donors into CPDA-1. Extended storage of blood in blood banks lead to changes in biochemical and hematological parameters of stored blood. RBC stored for a period of time at 4°c loses viability. The hematological parameters, there was constant decline in WBC and platelet counts from day 0 to 28.RBC count, Hb, MCV, HCT showed increasing values. MHC was almost constant, while MCHC decreased. PDW increased while PCT increased till 4 day and then decreased. Granulocytes decreased while lymphocytes increased. Among the biochemical parameters, values of S. Sodium decreased, S. Chloride decreased till 3rd day, increased on 4th day and then again decreased on 5th day. S. Potassium and albumin showed increasing values (Bhargava., et al, 2016).

Study conducted in UK that demonstrated that changes to several haematological and biochemical parameters occur during the storage of blood, 5 units of whole blood suspended in SAGM additive solution was acquired from the NHSBT, United Kingdom (UK). Units were stored at standard blood bank

conditions (2-6°C) and analysed on days 1, 7, 14, 21 and 28. There were significant changes in mean cell volume (MCV) during the storage period ($p < 0.001$, ANOVA). Specifically, significant increases between day 1 storage versus days 7, 14, 21 and 28 ($p< 0.05$) were reported. Similarly, the haematocrit (Hct), an indicator of plasma cell volume, demonstrated significant increases during storage ($p < 0.001$). Compared with day 1 storage, significant increases were observed versus days 7 ($p= 0.028$), 14 ($p = 0.04$), 21 ($p = 0.008$) and 28 ($p= 0.030$). However, no significant changes were reported for red blood cell (RBC) levels during storage ($p= 0.526$) Total white cell count (WBC) count significantly decreased from day 1 to day 28 ($p = 0.026$) (Tongwiis and Hunghes, 2016).

A prospective cross sectional study designed to detect effect of storage duration on some hematological parameters at Elnehoud Teaching Hospital Blood Bank in Western Kordofan State. Collected blood from 50 healthy volunteer donors into CPDA-1. The study showed significant decrease in all parameters under study include ;Hemoglobin concentration g/dl, Red blood cells count, White blood cells count Hematocrit, Mean cell volume, Mean cell Hemoglobin, Mean cell Hemoglobin concentration and Platelets count (Breama, 2015).

The study was conducted in Korea to systematically investigate changes in stored whole blood, the hematological properties of individual RBCs were quantified in blood samples stored for various periods with and without a preservation solution called CPDA-1. the morphological (cellular volume, surface area, and sphericity), biochemical (Hb content, and Hb concentration) and mechanical properties (membrane fluctuation) of individual RBCs stored with and without CPDA-1 were systemically quantified. The results show no significant changes in cellular volumes, Hb contents and Hb concentrations during 6 weeks of storage for RBCs stored without CPDA-1. However, the surface areas of the RBCs significantly decreased during the first two weeks, showing sphericity values of

unity. The morphologies of the RBCs dramatically transformed in less than 1 week of storage. In the absence of CPDA-1, 60% of the RBCs stored without CPDA-1 were a non-discocyte shape at day 5; after day 13, all the RBCs became spherical shapes. These results the decrease in cell membrane area and the increase in sphericity imply that spherocytosis is induced by vesiculation in the stored RBCs. This storage-induced spherocytosis seems to cause a significant decrease in cell deformability; membrane fluctuations in the RBCs decreased by 53% after two weeks of storage (Park *et al*., 2015).

The study was conducted in Nigeria to evaluate of the effect of blood storage on both hematological and biochemical parameters was carried out using Citrate Phosphate Dextrose Adenine (CPDA -1) anticoagulated blood drawn from ten healthy volunteer donors and placed on the quarantine shelf of the blood bank refrigerator. The blood was kept for 28 days and samples were evaluated on days 1, 7, 14, 21 and 28. MPV and LPCR showed drastic increase from day 14 down to day 28. A progressive increase in RDW was noticed in this study, Other hematological parameters remained fairly stable during this study period (Adias *et al.,* 2012).

# CHAPTER III

# 3 MATERIAL AND METHOD

## 3.1 Methods:

### 3.1.1 Study design:

This is analytical study.

### 3.1.2 Study area and duration:

The study was conducted at Umdownban Hospital in East Nile Locality- Khartoum State during March to December 2019.

### 3.1.3 Study population:

Forty volunteer healthy donors in Umdownban Hospital

## 3.2 Inclusion criteria and exclusion criteria:

### 3.2.1 Inclusion criteria:

Donors who satisfied the blood donation criteria for blood donor selection of WHO (WHO,2012).

### 3.2.2 Exclusion criteria:

Donors who did not satisfy the blood donation criteria for blood donor selection of WHO (WHO,2012).

### 3.3 Methodology:

#### 3.3.1 Principle of Blood grouping:

Agglutination of red blood cells with a specific anti-sera indicates the presence of the corresponding Ag on the RBC and is interpreted as a positive test. Absence of agglutination indicates the corresponding Ag is not present.

##### 3.3.1.1 Reagent:

Monoclonal anti-A,anti-B,anti-D.

#### 3.3.2 Blood cell counts and indices:

Blood cell counts and indices were determined by three parts differential auto Hematological Analyzer-Mindray BC3000 Plus with large and high resolution colour LCD and printout via internal thermal printer. It determines 19 parameters and three histograms for WBCs, RBCs and platelets. The parameters include: WBC, Lymphocytes, Granulocytes, RBCs, HGB HCT, MCV, MCH, MCHC, Platelet, MPV, PDW and PCT.

##### 3.3.2.1 Principle:

Electrical impedance method for counting and cyanide free method light absorbance for measuring hemoglobin.

##### 3.3.2.2 Reagents:

Diluents.

Lysing reagent.

Rinsing/auxiliaries.

Emergency cleaning.

Hematology control.

### 3.3.3 Quality control:

Automatic diluting,lyzing,mixing,rinsing and unclogging and automatic monitoring of reagent status. Fully automatic calibration. Unique fresh blood calibration program.

### 3.4 Sample:

Anticoagulated whole blood was taken from stored bag in day one, day fifteen and day thirty.

### 3.5 Data collection:

Demographic data of the participant were collected by a questionnaires which was specifically designed for this study.

### 3.5.1 Data analysis:

ANOVA test was performed for determining the effect of storage time on CBC and data were statistically checked and analyzed using Statistical Package of Social Science (SPSS) version 20.

### 3.6 Ethical consideration:

The study was approved by Ethical and Scientific Committee, Medical Laboratory Science College-Sudan University of Science and Technology and Omdawnban hospital. Verbal consent from the donors was taken at the beginning of the study. Confidentiality of the donor's names and the results was maintained and the blood samples will not be used for any other purpose than of this research.

# CHAPTER IV

# 4 RESULTS

## 4.1 Results:

### 4.1.1 Frequency of donor's blood groups:

**Figure (1):** The Distribution of the study population according to their blood type was Blod group O Rh (55%) >group A Rh positive (20%) > group B Rh positive (15%)> AB Rh positive (zero). The rest of the donors (10%) were Rh negative and their distribution was (2.5%) for each of the four blood groups.

### 4.1.2 The effect of duration storage on RBCs Count, Hb and HCT:

**Table (4.1)** Shows that storage of the blood did not have any significant ($P \leq 0.05$) effect on RBCs, Hb, or HCT. The highest mean RBCs count is ($4.68 \times 10^{12} \pm 0.66$ /L) in day one and the lowest mean is ($4.52 \times 10^{12} \pm 0.84$/ L) in day thirty. The highest mean Hb concentration is (12.5 g/dl±1.56 g/dl) in day one and the lowest mean is (12.0±1.96 g/dl) in day thirty. The highest HCT mean value is (43.2 ±9.91%) in day thirty and the lowest mean is (39.4±7.51%) in day one.

### 4.1.3 The effect of duration of storage on RBCs indices and RDW:

**Table (4.2):** MCV was significantly (P=0.000) increased with the length of the storage period, the highest mean (97.2±9.55fL) was registered in day thirty and the lowest value was (86.5±6.14 fL) in day one. MCH was not affected by storage. MCHC of day one (31.0±1.31g/dl) was significantly (P=0.000) higher than the values of day fifteen (28.8±3.24 g/dl) and day thirty (27.2±3.11g/dl).A significant (P=0.000) increase in RDW.CV was registered in day fifteen and day thirty and insignificant increase was found between the two storage periods. RDW.SD

showed a significant (P=0.014) increase between day one and day thirty. Insignificant increases between day one and day fifteen, also insignificant increases between day fifteen and day thirty. The highest mean is (51.7±6.79 fL) in day thirty and the lowest mean is (48.0±5.30fL) in day one.

**4.1.4 The effect of duration storage on leukocyte count and differential count:**

This is displayed in (Table 4.3**).** White blood cells count was significant decreased. The highest mean of WBCs count is (4.9±1.50×$10^9$ cell/L) in day one and the lowest mean is (3.6±1.37× $10^9$cell/ L) in day thirty. No significant (P≤ 0.05) differences were found between day fifteen and day thirty. Lymphocytes count was significantly (P=0.000) increased with the storage and the highest mean was (73.6±17.65%) in day thirty and the lowest mean is (37.8±13.20%) in day one. Granulocytes count was significantly (P=0.000) decreased periods, the highest mean of granulocyte percent is (48.9±11.66%) in day one and lowest mean is (26.5±14.78%) in day fifteen with significant increase between day fifteen and day thirty. The result of Mid percent was in significant (P=0.111).

**4.1.5 The effect of duration storage on platelet count and platelet indices:**

**Table (4.4):** The result of Platelet count was significantly decreased with the length of the storage periods. The highest mean is (202±47.9× $10^9$ cell/L ) in day one followed by (152±67.2×$10^9$ cell/L) in day fifteen and the lowest mean is (121±54.2× $10^9$cell/ L) in day thirty. The highest mean of PDW is (16.5±1.55) of day thirty is significantly (P=0.001) higher than (15.0±2.0) of day one. Day thirty registered significantly (P=0.000) lower mean PCT value than day one. No significant variation was found between day fifteen and day thirty in the values of PDW or PCT. MPV result was not affected significantly (P≤ 0.05) with the storage periods.

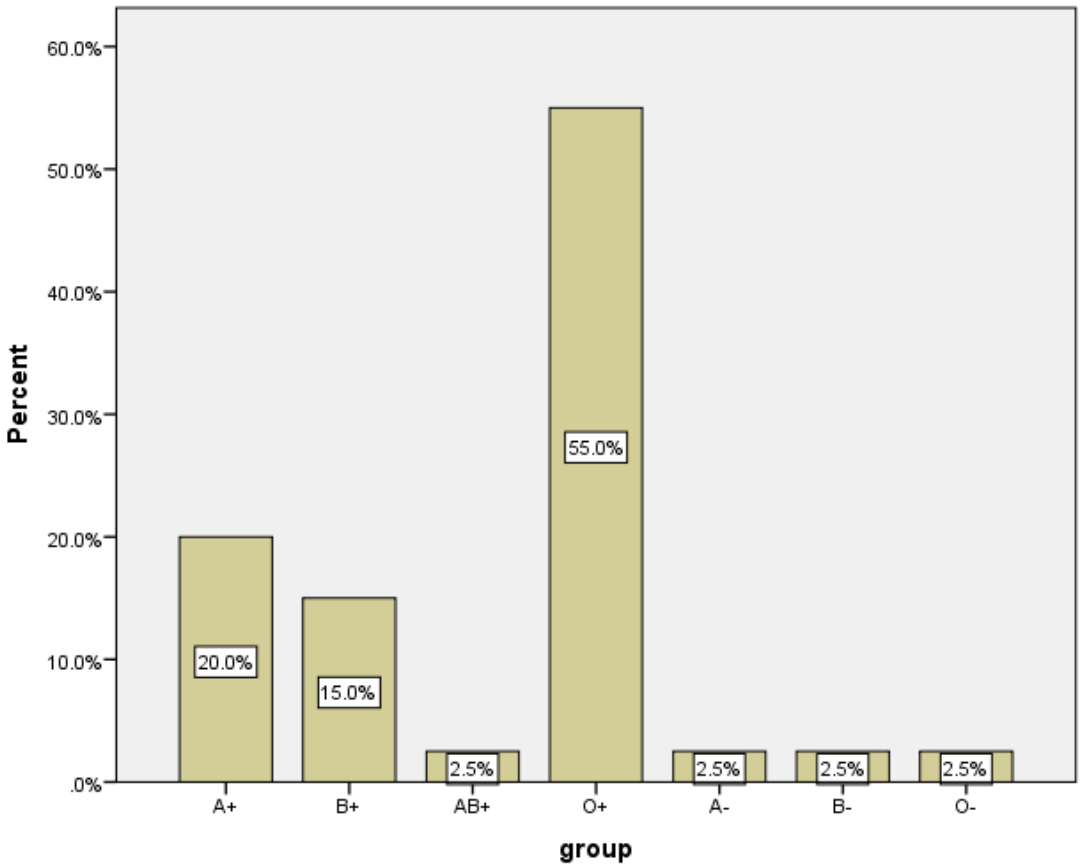



**Figure 4.1: frequency of donor's blood groups**

**Table (4.1): The effect of duration storage on RBCs Count, Hb and HCT:**

| Days | RBCs$\times 10^{12}$/ L (Mean±SD) | HGB g/dl (Mean±SD) | HCT% (Mean±SD) |
|---|---|---|---|
| Day 1: | 4.68±0.66 | 12.5±1.56 | 39.4±7.51 |
| Day 15: | 4.60±1.08 | 12.2±2.81 | 41.8±11.11 |
| Day 30: | 4.52±0.84 | 12.0±1.96 | 43.2 ±9.91 |
| P.Value | 0.735 | 0.575 | 0.202 |

**Table (4.2): The effect of duration storage on RBCs indices and RDW:**

| Days | MCV fL (Mean±SD) | MCH Pg (Mean±SD) | MCHC g/dl (Mean±SD) | RDW.CV% (Mean±SD) | RDW fL (Mean±SD) |
|---|---|---|---|---|---|
| Day 1: | 86.5±6.14$^{c}$ | 26.6±2.46 | 31.0±1.31$^{a}$ | 14.2±1.16$^{b}$ | 48.0±5.30$^{b}$ |
| Day 15: | 90.8±11.39$^{b}$ | 26.7±3.09 | 28.8±3.24$^{b}$ | 15.0±1.11$^{a}$ | 49.9±4.30$^{ab}$ |
| Day 30: | 97.2±9.55$^{a}$ | 27.1±3.17 | 27.2±3.11$^{c}$ | 15.3±1.12$^{a}$ | 51.7±6.79$^{a}$ |
| P.Value | 0.000 | 0.649 | 0.000 | 0.000 | 0.014 |

*a,b,c mean within the same column with different superscripts was significantly different

at P. $\leq$ 0.05.

**Table (4.3): The effect of duration storage on leukocyte count and differential count:**

| Days | WBCs× $10^9$ /L (Mean±SD) | Lymphocyte% (Mean±SD) | Mid% (Mean±SD) | Granulocyte% (Mean±SD) |
|---|---|---|---|---|
| Day 1: | 4.9±1.50[a] | 37.8±13.20[c] | 11.9±4.72 | 48.9±11.66[a] |
| Day 15: | 4.0±1.46[b] | 60.5±21.80[b] | 10.9±7.12 | 26.5±14.78[c] |
| Day 30: | 3.6±1.37[b] | 73.6±17.65[a] | 8.8±7.57 | 30.3±18.27[b] |
| P.Value | 0.000 | 0.000 | 0.111 | 0.000 |

*a,b,c mean within the same column with different superscripts was significantly different

at P. ≤ 0.05.

**Table (4.4): The effect of duration storage on platelet count and platelet indices:**

| Days | PLT× $10^9$/ L (Mean±SD) | MPV fL (Mean±SD) | PDW (Mean±SD) | PCT% (Mean±SD) |
|---|---|---|---|---|
| Day 1: | 202±47.9[a] | 7.6±0.76 | 15.0±2.0[b] | 0.14±0.04[a] |
| Day 15: | 152±67.2[b] | 8.0±1.20 | 15.7±1.61[ab] | 0.12±0.05[ab] |
| Day 30: | 121±54.2[c] | 7.9±0.68 | 16.5±1.55[a] | 0.09±0.04[b] |
| P.Value | 0.000 | 0.168 | 0.001 | 0.000 |

*a,b,c mean within the same column with different superscripts was significantly different

at P. ≤ 0.05.

# CHAPTER V

# 5 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 5.1 Discussion:

The storage of whole blood did not cause any significant changes in RBCs count, hemoglobin levels or HCT. The highest means of RBCs count is $4.68\times10^{12}$ /L in day one and the lowest mean is $4.52\times10^{12}$/ L in day thirty. Also insignificant changes in Hemoglobin level among the three storage periods. The highest mean is 12.5 g/dl registered in day one and the lowest mean is 12.0 in day thirty. HCT showed an insignificant increase among the three periods. The highest mean is 43.2% in day thirty and the lowest mean is 39.4% in day one. These findings accord with the study conducted by Adias *et al* (2018) in Nigeria in which they investigated the effect of storage on haematological changes of CPDA-1 whole blood. Park *et al*., (2015) in Korea investigated changes in stored whole blood with and without CPDA-1 and they did not find any significant changes in the hemoglobin level, cell concentration or volume in both specimens that is with or without addition of CPDA-1 .There are previous studies which showed significant changes (decrease or increase) in the values of RBCs, Hb and HCT. A significant decrease in RBCs, Hb and HCT was found by Breema (2015) in Sudan and in Pakistan by Asif zeb.,*et al.*, (2016). Storage caused an increase in Hb, HCT and RBCs count according to two earlier studies performed in India by Bhargava *et al* (2016) and in UK by Tongwiis and Hunghes, (2016) which contradicts the findings of this study.

RBCs indices values varied in their responses to storage and a discrepancy was observed in the findings of other researchers in the Sudan or abroad. The highest mean of MCV is 97 fL in day thirty and the lowest mean is 86 fL in day one. A significant increase in MCV values was registered, with the progress of storage,

this result is on line with the findings of Bhargava P., et al (2016), Tongwiis and Hunghes, (2016) and it contradicts the findings of Breema (2015), who found a significant decrease in MCV, and Asif zeb et al (2016) who did not observe any significant change in MCV. MCH was not affected by storage which is on line with the findings of Asif zeb.,et al., (2016) and disagree with Breema (2015) who found a significant decrease in MCH. MCHC was reduced with the length of the storage period which accords with Bhargava et al (2016). Storage increased the values of RDW.CV% and RDW.SD fl which is on line with the findings of Adias et al (2018).

The stability of the RBCs can be attributed to the use of CPDA-1 which contains citrate as anticoagulant and phosphate which keeps the pH low and dextrose which maintains the cell and adenine to maintain a constant ATP level throughout the storage period (Shield, 1969).The discrepancy between this work and some previous studies can be attributed to differences in the sample size, the preservation solution, handling or processing of the blood and or bacterial contamination that changes the environment. Also the age of the RBCs is another factor which may have caused this discrepancy as the aged cells undergo cellular changes faster than the younger ones. Damaged WBCs release many substances, like histamine, lipids which may interfere with the RBCs viability.

WBCs count and differential count showed a significant decrease, which is on line with various studies in this regard. The highest mean of WBC‘s count is $4.985\times10^{9}$/ L in day one and the lowest mean is $3.600\times10^{9}$/ L in day thirty. The change in WBCs was observed in day fifteen and it did not progress any more with the length of the storage period, in the contrary to the findings of Bhargava *et al* (2016) who recorded that WBC count is reduced subsequently from day one to day 28 and they suggested that the mechanism of leukocyte depletion and loss of viability during whole blood storage may be due to ATP depletion, aging and loss

of the cells characteristics like phagocytosis. Significant decrease in Granulocyte count, The highest mean is 48.9 % in day one and the lowest mean is 30.3 % in day thirty which is on line with Breama,(2015), Bhargava *et al* (2016), Asif zeb *et al* (2016) and Adias *et al* (2018). Lymphocyte showed a significant increase. The highest mean is 73.6% in day thirty and the lowest mean is 37.8% in day one, this result is on line with the findings of Bhargava *et al* (2016).

The Platelet count was significantly decreased with the progress of the storage period, this is on line with the findings of (Breama, 2015; Bhargava *et al*., 2016) .The highest mean is $202\times10^{9}$/ L in day one and lowest mean is $121\times10^{9}$/ L in day thirty. Kristoffersen and Apelseth (2019) reviwed the platelets functionality in cold –stored blood and they reported that some researcher found no change in the platelets count even at day 27 storage ,whereas others registered a decrease up to 50% ; and they attributed these variations to differences in pre-sampling manipulation. Other causes for the reduction in the platelets count were reported by Sahler *et al* (2011) that senescent platelets normally are cleared from the circulation by the liver and spleen but in a stored concentrates of platelets they remain and may react with other platelets and activate them. Also platelets may undergo apoptosis or die for other reasons. WBCs in whole blood produce substances which affect the physiology of the platelets leading to their destruction. The highest mean of PDW is 16.5 after thirteen storage period and lowest mean is 15.0 in day one. PDW result was showed significant increase similar to result of Bhargava *et al* (2016) and Adias *et al* (2018). The highest mean of PCT is 0.14% in day one and lowest mean is 0.09 in day thirty which is on line with Breama (2015). MPV result was showed insignificant changes this result differ from result of Adias *et al* (2018), result that was showed increases in MPV.

## 5.2 Conclusion:

From these results, the study concluded that:

-Storage period did not affect the mean values of Hb, HCT, RBCs count, MCH and MPV.

- Storage period increased the mean values of MCV and lymphocyte in day fifteen and day thirty while the increase in PDW was recorded in day thirty.

- After fifteen days storage WBCs were decreased and no significant decrease was found between day fifteen and thirty.

-Granulocytes and platelets count were decreased in day fifteen and the decrease continued till day thirty.

## 5.3 Recommendations:

Fresh blood should be given to the patients that need WBCs or platelets to avoid risk due to changes of blood during storage.

-Increase sample size by increase of donors to expanding the field.

- Before storage some of the donated blood should be separated to blood components to fit with the needs of the patients.

-More studies should be conducted to investigate the other probable morphological and biological changes of blood during storage.

## 5.4 References

**Adias, T. C.,** Moore-lgwe, B. and Jeremiah, Z. A. (2012). Storage Related Hematologyical and Biochemical Changes of CPDA-1 Whole Blood in a Resource Limited Setting, *Blood Disorders and Transfusion.* **3** (3).

**Alessandro, A.,** Liumbruno, G., Grazzini, G and Zolla, L. (2010). Red Blood Cell Storage: The Story So Far, *Blood Transfusion*. **8**. P: 82-88.

**Ali, N. T.** (2017). Effect of Storage at Temprature (4°C) on Complete Blood Count Parameters, *Journal of Cancer Treatment and Research.* **5** (2), P: 7-10.

**Andrew, P.,** Beckett, A., Benov, A., Borgman, M., Chan, J., Jason, B., Doughty, H., Fisher, A., Glassberg, E., Gonzales, R., Shawn, F., Wilbur, W., Nessen, S., Jeremy, G., Prat, Quesada, J., Reade,M., Sailliol, A., Philip, C., Stockinger, Z., Strandenes, G., Taylor, A.,Yazer, M., Bryant, B and Gurney, J.(2018). Whole Blood Transfusion. *Military Medicine*. **183**. P: 44-51.

**Asif zeb, M.,** Purnoor, F., Mansoor M., Saidal, A and Waheed, S. **(**2018). Effect of Storage Time on Hematological and Biochemical Parameters in Blood Bags Containing CPDA-1 Anticoagulant during 30 Days of Storage, *Indo American Journal of Pharmaceutical Sciences*. **05** (10), P: 10522-10526.

**Bashi, A. D** and Saleh, B. M. (2009). Effect of Blood Storage on Certain Hematological Parameters, *Tikrit Medical Journal.* **15**(1), P: 17-180.

**Bhargava, P.,** Gupta, R and Khare, V**.** (2016). Stored Blood Induced Effect Hematological and Biochemical Parameter up to 28 Days, *Recent Advances in Pathology Laboratory Medicine*. **2** (34), P: 8-12.

**Breama, S. B.** (2015). Effect of Storage Duration on Some Hematological Parameters of CPDA-1 Whole Blood at Elnehoud Teaching Hospital-Westeren

Kordofan. (Unpublished Master‘s dissertation),SUST Repository. Khartoum-Sudan.

**Brecher, M. E.** (2005). Technical Manual Program Unit, 15thed, USA: AABB.

**Briggs, C.** and Bain, B. J. (2011). Basic Hematological Techniques. In: Bain, B.J., Bates F., Laffan M. A and Lewis S. M. Dacie and Lewis Practical Hematology, 11thed, China: E-book. Elsevier Health Sciences: Churchill livingstone.

**Budak, Y. U.,** Polat, M and Huysal, K. **(**2016). The Use of Platelet Indices, Plateletcrit, Mean Platelet Volume and Platelet Distribution Width in Emergency Non-Traumatic Abdominal Surgery: A systematic Review. *The journal of Croation Society of Medical Biochemistry and Laboratory Medicine*. **26** (2), P: 178-193.

**Cheesbrough, M.** (2006). District Laboratory Practice in Tropical Countries, 2nded, United States of America: Cambridge University Press, New York.

**Ciesla, B**. (2007). Hematology in Practice 1st ed, USA: F.A Davis Company.

**Domen, R. E** and Hoeltge, G. A. (2003). Allergic Transfusion Reactions, *Archives Pathology and Laboratory Medicine*. 127, P: 316-320.

**Eldad, A.,** Hod., Zhang, N., Set A., Sokol., Boguslaw S., Wojczyk., Richard O., Franceis., Ansaldi, D., Kevin P., Franceis., Della-Latta, P., Whittier, S., Sheth, S., Jeanne E., Hendrickson., Zimring, J. C., Gary, M., Brittenham and Spitalnik, S. L. (2010). Transfusion of Red Blood Cells after Prolonged Storage Produces Harmful Effects That are Mediated by Iron and Inflammation. *Blood journals, The American society of hematology.* **115**(21), P: 4284-4292.

**Firkin, F.,** Chesterman, C., Penington, D and Rush, B. (2006). de Gruchy‘s Clinical Hematology in Medical Practice. 5th ed. India: Gopsons papers LTD**.**

**Greening, D. W.,** Glenister, K. M., Sparrowb, R. L and Simpson, R. J. (2009). International Blood Collection and Storage: Clinical Use of Blood Products, *Journal of Protemics*. (73), P: 386-395.

**Greer, J. P.,** Arber, D.A., Glader, B., List, A. F., Means, R.T., Poraskevas, F., Rodgers, G. M and Foerster, J. (2014). Wintrobe's Clinical Hematology.13th ed. Philadelphia: Lippincott Williams & Wilkins.

**Hardwick, J.** (2008). Blood Storage and Transportation. *ISBT Science Series*. **3**. P: 177-196.

**Harmening, D.M .** (2005). Modren Blood Banking and Transfusion Practices, 5th ed, Philadelphia:A.Davis company.

**Hoffbrand, A. V.,** Moss, B. A and Pettit J.K. (2006). Essential Hematology.5th ed .Oxford: Blackwell.

**Hoffman, R.,** Edward J., Shattil,S. J., Furie, B., Cohen, H. J., Silberstein, L. E and McGlave P. (2000). Ronald Hoffman. Hematology: Basic Principle and Practice,3rd ed, USA: Charchill Livingstone Inc.

**Key, N.,** Makris, M., O'Shaughnessy, D., Lillicrap, D. (2009). Practical Hemostasis and Thrombosis, 2nd ed. UK: Blackwell Puplishing LTD.

**Kristoffersen**, **E. I** and Apelseth, T. O. (2019) Platelets Functionality in Cold – Stored Blood**.**
14, (3) P: 308-314.

**Lokwani, D. P**. (2013). The ABC of CBC Interpretation of Complete Blood Count and Histogram,1st ed, India: Japee Brothers Medical Publishers.

**Maniatis, A.,** Linden P.V and Hardy J.F. (2011). Alternatives to Blood Transfusion in Transfusion Medicine,2nd ed, Blackwell Publishing Ltd.

**Marchant, K.K** and Davis, B.H. (2012). Laboratory Hematology Practice.1st ed, UK: Black Well Publishing Ltd.

**McKenzie, S.** and Williams, J. (2010). Clinical Laboratory Hematology, 2nd ed, Pearson.

**Meledeo,** M. A., Peltier, G. C., McIntosh, C. S., Bynum J. A. and Cap A. P. (2019). Optimizing Whole Blood Storage: Hemostatic Function of 35-Day Stored Product in CPD, CP2D, and CPDA-1 Anticoagulants. *Transfusion.* (59), P: 1549-1559.

**Nayak, R.,** Rai, S. and Gupta, A. **(**2012). Essentials in Hematology and Clinical Pathology, 1st ed, India: Jaypee Brothers Medical Publishers..

**Ozment, C. P.** and Turi, J. L. (2009). Iron Overload Following Red Blood Cell Transfusion and its Impact on Disease Severity, *Biochemica et BiophysicaActa (BBA)-General Subjects*,1790 (7), P: 694-701.

**Park, H.,** Misuk, J., Lee, S., Kim, K., Sohn, Y.H., Jang S. and Park Y. (2015). Alterations in Cell Surface Area and Deformability of Individual Human Red Blood Cells in Stored Blood. Korea.[Online]Available from; https//www.semanticscholar.org/paper/Alterations-in-cell-surface-area-and-deformability-Park-Ji/7e546d52463a86b9e035b1fdb4679e3804259886, [Accessed;21March 2020].

**Queen, E.,** Ifeanyi, O.E and Chiedum, O.K. (2014). The Effect of Storage on Full Blood Count in Different Anticoagulant. *IOSR Journal of Dental and Medical Sciences*. 13(9). P: 128-131.

**Sahler, J**., Grimshaw, K., Spinelli, S L., Refaai, M. A., Phipps, R. P and Blumberg N. (2011) Platelet Storage and Transfusions: New Concerns Associated with an Old Therapy. *Drug discovery today. Disease mechanisms* 8,P: 1-2.

**Sahu, S.,** Hemlata and Verma, A. (2014). Adverse Events Related to Blood Transfusion, *Indian Journal of Anaesthesia*. **58**(5), P: 543-551.

**Shaz, B. H.,** Hillyer,C D., Roshal, M and Abrames, C. (2013). Transfusion Medicine and Hemostasis, Clinical and Laboratory Aspects. 2nded, USA: Elsevier Inc.

**Shields C. E.** (1969) Effect of Adenine on Stored Erythrocytes Evaluated by Autologous and Homologous Transfusions. *Transfusion* 9, P: 115-119.

**Sohi, I** and Jacob, S. (2005). Transfusion Associated GVHD, *Indian Journal of Pediatrics*. 72(6), P: 533-535.

**Sparrow, R. L.** (2012). Time to Revisit Red Blood Cell Additive Solutions and Storage Conditions: a role for "omics" Analyses, *Blood Transfusion*. (10), P: 7-11.

**Tenorio, G. C.,** Gupte, S. C and Munker, R. (2007). Transfusion Medicine and Immunohematology.In: Munker, R., Hiller E., Glass J and Paquette R. Modren Hematology Biology and Clincal Management,2nded, USA: Humana Press Inc.

**Tongwiis, P. E.** and Hunghes, S.F. (2016). The Effect of Storage on Various Haematological and Biochemical Parameters of NHSBT Donor Blood, *Jacobs Journal of Hematology*. **2** (2).

**Treleaven, J.,** Gennery, A., Marsh, J., Norfolk, D., Page, L.,Parker, A., Saran, F., Thurston, J and Webb, D.(2010). Guidelines on The Use of Irradiated Blood Components Prepared by The British Committee for Standards in Haematology Blood Transfusion Task Force. *British Journal of Hematology*. **152**. P: 35-51.

**Turgeon, M. L. (**2012). Clinical Hematology: Theory and Procedures.5thed. Philadelphia: Lippincott Williams & Wilkins.

**Vörös, E.,** Madeleine L., Lezzar, D. and Shevkoplyas, S. S. (2018). Centrifugation-Free Washing Reduces Buildup of Potassium and Free Hemoglobin

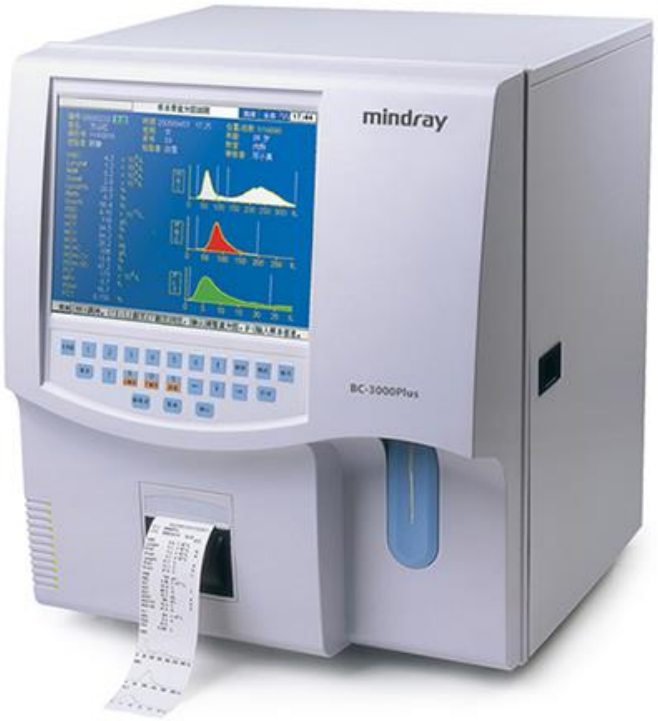
in Washed Red Blood Cells After The Procedure. *American journal of hematology*. **93**(12), P: E389-391.

**Whitlock, S. A.** (2010). Immunohematology for Medical Laboratory Technicians, 1st ed, USA: Delmar, Cengage Learning.

**William, F**. (2002). PDQ Hematology, 1st ed, Canada: BCDecer Inc.

**World Health Organization**.(2012). Blood Donor Selection: Guidelines on Assessing Donor Suitability for Blood Donation, USA: WHO.

## Appenex I:




**BC-3000 Plus**

**Auto Hematology Analyzer**

## AppenexII:

**بسم الله الرحمن الرحيم**

**QUESTIONNAIRE**

**Sudan University of Sciences and Technology**

**College of Graduate Studies**

**Eeffect of Storage Time on CBC in Whole Blood Bags Containing CPDA-1 Anticoagulant in khartoum state**

**Demographic data:**

Date: ……  Numper: ……...

Name: ……..  Age: ……...

Address…….....  Occubation: ….……

Education level: No ( ) primary ( ) secondary ( ) high ( ).

-are you feeling well today? Yes ( ) No ( ).

-have you donation blood? Yes ( ) Date: ……. No ( ).

-have you receive a blood transfusion? Yes ( ) Date: …….. No ( ).

-have you been under doctor care? Yes ( ) ……. No( ).

-Do you have currently have an infection? Or are you taking antibiotic for an infection? Yes ( ) Type: ……..No ( ).

-have you taken a drug? Yes ( ) Type: ……. No ( ).

-have you ever had heart problem, lung or bleeding problem?

Yes ( ) problem……. No ( ).

-have you had any vaccination? Yes ( ) Type: …… No ( ).

-are you feeling well (after donation)? Yes ( ) No ( ) ……

**Physical examination:**

B/p:

CBC result:

| Parameter/day | Day 0 | Day 15 | Day 30 |
|---|---|---|---|
| WBC‘s× $10^9$/ L | | | |
| Lymph#×$10^9$/ L | | | |
| Mid# ×$10^9$/ L | | | |
| Gran# ×$10^9$/ L | | | |
| Lymph % | | | |
| Mid % | | | |
| Gran % | | | |
| HGB g/dl | | | |
| RBC‘s×$10^{12}$/ L | | | |
| HCT % | | | |
| MCV fL | | | |
| MCH pg | | | |
| MCHC g/dl | | | |
| RDW.CV% | | | |
| RDW.SD fL | | | |
| PLT×$10^9$/ L | | | |
| MPV fL | | | |
| PDW | | | |
| PCT% | | | |

# CHAPTER I

# INTRODUCTION

# CHAPTER II

# LITERATURE REVIEW

# CHAPTER III

# MATERIAL AND METHOD

# CHAPTER IV

# RESULTS

# CHAPTER V

# DISCUSSION, CONCLUSION AND RECOMMENDATIONS